



The Effect of Irrigation Patterns on Flavonoid and Phenol Content in Mandau Sorghum Varieties

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ABSTRACT

This study analyzed the effect of drought stress on flavonoid and phenol content in Mandau sorghum varieties. The experiment used a completely randomized design to use four irrigation patterns (daily, every 4, 5, and 6 days). The results showed that drought stress had a significant effect on phenols but not on flavonoids. The highest flavonoid (580.0 µg/g) and phenol (2555.0 µg/g) contents were obtained with watering every 4 days, while the lowest flavonoid (395.0 µg/g) was obtained with watering every 5 days. Phenols increased with stress intensity, while flavonoids tended to decrease under severe stress. Mandau sorghum has potential as a source of bioactive metabolites for natural bioherbicides and sustainable agriculture

INTRODUCTION

Global climate change, characterized by rising temperatures and decreasing water availability, has led to more severe droughts, posing a serious challenge to the agricultural sector, especially for food crops highly dependent on water availability. Therefore, there is a need for commodities that are adaptive to abiotic stresses such as drought, one of which is sorghum (Khalifa & Eltahir, 2023). As a cereal crop, sorghum is known to be tolerant to marginal land and dry conditions, and has great potential as a source of food, animal feed, and bioenergy raw material (Hossain et al., 2022). In Indonesia, superior varieties such as Sorghum Mandau have been widely developed due to their high productivity and adaptability to various environments. However, sorghum's utilization has focused on seed production, while studies on its secondary metabolite content are relatively limited (Yustianisa & Diana, 2023).

Drought can trigger oxidative stress in plants, characterized by an increase in Reactive Oxygen Species (ROS) formation. In response, plants activate the synthesis of secondary metabolites, particularly flavonoids and phenols, which act as natural antioxidants to protect cells from damage caused by ROS. In addition to their role in defense mechanisms, these two groups of compounds also have potential applications as raw materials for pharmaceuticals, functional foods, and natural bioherbicides (Mohagheghian et al., 2025).

To date, most research on sorghum has focused on its morphological and physiological responses to drought. At the same time, studies on flavonoid and phenol content, particularly in the Mandau variety, remain very limited. However, this information is important for understanding the adaptation mechanisms of this superior variety to dry conditions, as well as opening up opportunities for the use of sorghum as a source of value-added bioactive compounds (Yue et al., 2025)

Based on this, this study aims to examine the effect of drought stress on the flavonoid and phenol content in Mandau variety sorghum extracts (Yue et al., 2025). The results obtained are expected to expand the use of sorghum, not only as a food commodity but also as a producer of secondary metabolites that support the development of sustainable agriculture and bio-based industries (Cunha et al., 2024).

LITERATURE REVIEW

Sorghum is a cereal crop resistant to dry climates and can thrive in marginal soils. During drought, secondary metabolites such as phenolics, flavonoids, and terpenoids increase. These metabolites protect the plant from pathogens, herbivores, and enemies (Akula & Ravishankar, 2011). One of the phenolic compounds produced by sorghum roots is sorgoleone. This compound acts as an allelopathic agent and stops the growth and germination of surrounding weeds. The production of sorgoleone and other secondary metabolites increases due to drought stress, strengthening sorghum's defense against competing plants. These biochemical changes are triggered by drought, which increases oxidative stress and activates enzymes involved in phenylpropanoid biosynthesis. This leads to higher concentrations of phenolic compounds, such as sorgoleone. As a result, sorghum becomes a better natural bioherbicide (Khan et al., 2020).

Sorghum fights weeds naturally, especially in dry environments prone to drought. Sorghum is useful as a natural weed killer (Weston & Czarnota, 2001). Sorghum helps control weeds in these areas and reduces the need for chemical herbicides, which can damage the environment. Thus, it should be noted that drought stress in sorghum increases the production of allelopathic secondary metabolites. These metabolites can be used as bioherbicides. Further research is needed to identify the specific active compounds responsible for these allelopathic effects and to determine how these compounds can be used in broader, sustainable, and environmentally friendly agricultural systems (Susilo et al., 2023; Susilo et al., 2024; Susilo et al., 2021). According to Akula and Ravishankar (2011), abiotic stress such as drought can increase the production of plant secondary metabolites, such as flavonoids. Flavonoids, natural antioxidants, protect plants from oxidative stress from the environment. Khan et al. (2020) found that drought activates enzymes responsible for the biosynthesis of phenylpropanoids, which produce flavonoids. Ferdinando et al. (2014) stated that flavonoids play an important role in protecting plant cells from damage caused by drought. Their production increases in response to environmental changes, making secondary metabolites important for plant adaptation. Phenolic acids, one example of phenolic compounds, have been globally recognized as allelopathic agents and have great potential to develop into environmentally friendly bioherbicides (Hoang Anh et al., 2021).

METHODOLOGY

This study used water extract from the Mandau sorghum variety obtained through several stages of treatment. The plants were grown on coastal land with four irrigation frequency treatments: daily, every four days, every five days, and every six days. Harvesting was carried out at 40 days of age, then the plants were separated into tops and roots. The materials were dried in the sun for 14 days, then in an oven at 70°C for 72 hours. The dried samples were then cut into 1–2 cm pieces, ground into powder using a blender, and used as material for making water extracts to analyze flavonoid and phenol content.

Flavonoid content analysis was performed by dissolving 10 mg of extract in 10 ml of solvent. From this solution, 0.5 ml was taken, then 1.5 ml of 96% ethanol, 0.1 ml of 10% AlCl₃, 0.1 ml of 1 M CH₃COONa, and 2.8 ml of distilled water were added. The mixture was incubated for 30 minutes, then measured at a wavelength of 435 nm with a similarly treated standard series. Phenol content analysis was performed by dissolving 10 mg of extract in a 10 ml volumetric flask, then taking 0.2 ml of the solution and adding 15.8 ml of distilled water. Next, 1 ml of Folin-Ciocalteu reagent was added, incubated for 8 minutes, then 3 ml of 20% Na₂CO₃ was added and left for 30 minutes. Absorbance was measured at a wavelength of 765 nm with a similar procedure for the standard series.

The main variables observed in this study were flavonoid and phenol content. The study used a one-factor completely randomized design (CRD) with four replicates. The observation data were analyzed using ANOVA, and if there were significant differences, they were followed up with a BNT test at a significance level of 5% ($P < 0.05$).

RESULTS AND DISCUSSION

The variables observed in this study included flavonoid and phenol levels in the extracts. The results of the analysis of variance (Table 1) showed that treating Mandau sorghum water extracts with different levels of drought stress had a very significant effect on phenol levels but not on flavonoid levels.

Table 1. Recapitulation of the Variance Analysis of Flavonoid and Phenol Levels

Observation variables	Treatment
Flavonoid	2.64 tn
Fenol	33.03 **
* = significant effect	
N = no significant effect	

The effect of treatment with extract from the Mandau variety of sorghum showed no significant effect on flavonoid content. There is a tendency that the highest flavonoid content was achieved by watering every 4 days (580.0 $\mu\text{g/g}$) and the lowest flavonoid content was achieved by watering every 5 days (395.0 $\mu\text{g/g}$), as shown in Table 2. This study shows that watering every day to every 4 days produces low and high flavonoid content. Furthermore, the treatment of watering every 5 days resulted in a decrease in flavonoid levels. This shows that the higher the level of drought stress, the higher the flavonoid levels, but this is limited to the stress level with watering every 4 days. The 5-day irrigation pattern showed the lowest flavonoid levels, 395.0 $\mu\text{g/g}$.

The pattern of flavonoid response to drought stress generally forms a curve: flavonoid levels increase under mild to moderate stress (watering every 4 days), then decrease under severe stress (watering every 5–6 days). During the mild to moderate drought phase, the phenylpropanoid pathway (PAL, CHS, CHI) is activated, thereby increasing flavonoid synthesis to neutralize excess ROS. At 5-day intervals, stress signals such as ABA and H_2O_2 are strong enough to promote biosynthesis, but do not yet interfere with the plant's metabolic system.

Conversely, during severe drought, stomatal closure causes a decrease in photosynthesis, low CO_2 availability, and a reduction in ATP and NADPH reserves. This limits the supply of carbon (phenylalanine, malonyl-CoA) and energy needed for flavonoid formation. Under these conditions, excess ROS damages membranes and enzymes important to the flavonoid pathway. At the same time, metabolic resources are directed more toward vital functions such as osmoregulation and cell damage repair than toward the production of secondary metabolites. Extreme drought even triggers leaf senescence, characterized by chlorophyll degradation and oxidizing secondary metabolites; some flavonoids can be used as “ROS sinks” or relocalized, resulting in decreased measurable levels.

Additional factors such as leaf phase differences (older or beginning to wilt), low tissue water content affecting extraction efficiency, and data normalization methods (based on fresh or dry weight) can also make flavonoid values appear lower under severe stress.

In line with this, Kustiawan & Kusuma, (2019) reported that drought can increase flavonoid production as an adaptive mechanism. Zhang & Cheng, (2021) confirmed that abiotic stress, particularly drought, stimulates the accumulation of secondary metabolites, including flavonoids, for protection. In addition, [9] stated that flavonoids play an important role in plant resistance to stress by functioning as antioxidants, UV protectors, and signal regulators, so their production increases under stressful conditions.

Table 2. Average Flavonoid and Phenol Content of Mandau Sorghum Extracts Under Different Levels of Drought Stress

Watering Treatment	Flavonoid (µg/G)	Fenol (µg/G)
Every day	415.00	1085.0 c
Every 4 days	580.00	2555.0 a
Every 5 days	395.00	1560.0 b

Note: Numbers Followed by the Same Letter in the Same Column are not Significantly Different in the 5% LSD Test

The effect of treatment with extract from the Mandau variety of sorghum significantly affected phenol content. The irrigation pattern achieved the highest phenolic content every 4 days (2555.0 µg/g). It significantly differed from the daily irrigation treatment and the irrigation pattern every 5 days, as shown in Table 2. This study shows that daily to every 4 days irrigation produces phenolic content from low to highest. This indicates that as the level of drought stress increases, the phenolic content also increases. Subsequently, there was a decrease in phenolic content in the irrigation pattern every 5 days.

Phenols are adaptive secondary metabolites that play an important role in plant defense mechanisms against oxidative stress. Under normal conditions (daily watering), phenol levels are relatively low because ROS production is minimal and phenylalanine ammonia-lyase (PAL) enzyme activity has not been induced. When plants experience mild to moderate drought (watering every 2–4 days), partial stomatal closure reduces photosynthesis. It triggers the formation of moderate amounts of ROS, thereby increasing phenol synthesis as a cell protector. Under more severe stress (watering once every 5 days), higher ROS accumulation promotes increased PAL activity and accelerates phenol biosynthesis, which functions as a powerful antioxidant and a secondary osmoregulator. Even under the most severe stress (watering once every 6 days), although the biosynthesis of other metabolites tends to decrease due to energy limitations, phenol production continues to increase because the phenylpropanoid pathway is closely related to the plant's basic defense system as a stress-induced compound.

Phenol levels show a linear increase pattern with drought stress intensity because the continuous increase in ROS becomes a strong signal to activate the phenylpropanoid pathway. Unlike flavonoids, phenols are more "constitutively adaptive," so their production continues under severe stress conditions. This confirms that plants prioritize phenols as the main defense compound in extreme conditions. The increase in phenol levels from daily watering to once every 6 days reflects a consistent response to oxidative stress through increased phenol

synthesis as an antioxidant and cell protector. Previous studies support these findings. For example, drought stress has been shown to increase total phenolic content and antioxidant enzyme activity in barley by activating the phenylpropanoid pathway. Mohagheghian et al., (2025) ; Susilo et al., (2021); Sharma et al., (2019) reported that drought triggers phenolic accumulation by upregulating key enzymes in the phenylpropanoid pathway, such as PAL and CHS. Park et al., (2023) also showed that the accumulation of phenolic acids and flavonoids is a form of plant adaptation to drought stress through modulation of the phenylpropanoid pathway. Furthermore, Aluko et al., (2021) revealed that key genes such as PAL, C4H, 4CL, CHI, and F3H are induced under abiotic stress conditions. At the same time, Rao and Zheng (2025) confirmed that phenylpropanoid metabolism activity plays an important role in producing polyphenols that function as antioxidants, membrane stabilizers, and osmotic regulators to support plant resistance.

CONCLUSION AND RECOMMENDATION

The results showed that drought stress treatment with a four-day irrigation pattern produced the highest flavonoid content (580.0 g/g). The lowest flavonoid content was obtained in the five-day irrigation treatment (395.0 g/g). For the phenol parameter, the highest level was also achieved in the four-day irrigation treatment, at 2555.0 g/g. Flavonoid and phenol content generally increased with drought stress levels, up to a certain limit. These findings indicate that sorghum extracts from drought stress under certain conditions have the potential to be the best candidates as sources of natural bioherbicides.

FUTHER STUDY

This research still has delays, so it is necessary to conduct further research related to the topic The Effect of Irrigation Patterns on Flavonoid and Phenol Content in Mandau Sorghum Varieties in order to improve this research and add insight for readers.

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